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Sex estimation of the tibia in modern Turkish: a Computed Tomography study

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Abstract

The utilization of computed tomography is beneficial for the analysis of skeletal remains and it has important advantages for anthropometric studies. The present study investigated morphometry of left tibia using CT images of a contemporary Turkish population. Seven parameters were measured on 203 individuals (124 males and 79 females) within the 19–92-years age group. The first objective of this study was to provide population-specific sex estimation equations for the contemporary Turkish population based on CT images. A second objective was to test the sex estimation formulae on Southern Europeans by Kranioti and Apostol [4]. Univariate discriminant functions resulted in classification accuracy that ranged from 66 to 86 %. The best single variable was found to be Upper epiphyseal breadth (86%) followed by Lower epiphyseal breadth (85%). Multivariate discriminant functions resulted in classification accuracy for cross-validated data ranged from 79 to 86%. Applying the multivariate sex estimation formulae on Southern Europeans (SE) by Kranioti and Apostol in our sample resulted in very high classification accuracy ranging from 81 to 88%. In addition, 35.5-47% of the total Turkish sample is correctly classified with over 95% posterior probability, which is actually higher than the one reported for the original sample (25-43%). We conclude that the tibia is a very useful bone for sex estimation in the contemporary Turkish population. Moreover, our test results support the hypothesis that the SE formulae are sufficient for the contemporary Turkish population and they can be used safely for criminal investigations when posterior probabilities are over 95%.

Key words: Forensic Anthropology, Tibia, Sex estimation, CT-scans, posterior probabilities,

Introduction

Morphometric and morphological analyses of skeletal remains are very important to determine sex when fingerprints and DNA cannot be obtained. Previous anthropological studies have reported that the most accurate sex estimation methods are based on the pelvis and the cranium [1-3]. Despite the advantages of these two skeletal regions for sex estimation, they are not always available in all forensic and archeological investigations. Only one part of skeleton or parts of bones may be resources for researchers as a result of the type and severity of trauma before or after death, geographic factors, and secondary factors associated with decomposing human remains [1-3]. Therefore, long bones, particularly the femur and tibia with thick cortical structures and a wide volume, are more robust than other long bones and are useful for sex estimation [1-5]. In addition, new research supports that several postcranial elements are actually better indicators of sex compared to the cranium [2]. In this vein, many population-specific studies have investigated the utility of the tibia as an indicator of sex and the accuracy of sex estimation when using the tibia as assessed by several morphometric parameters is over 84% [6-17]

According to the religion of Islam, when a person dies, the first stage of the afterlife starts in the grave. Therefore, the use of dead bodies for scientific study purposes other than legal obligations is often impossible. In addition, prior to 1923, access to archival information of burials has been a challenge for researchers because the death records are written in the Ottoman language-Arabic alphabet and usually records of the Ottoman Empire period were not available [18]. Currently, researchers are in need of contemporary anthropological data for identification since a large number of mass graves have been found recently and there is a lack of anthropometric data in Turkey. The Human Rights Association has prepared a map of the locations of the verified mass graves [19]. According to the report released on 2014, 348 mass graves were recorded containing the remains of 4201 people since 1989 and these individuals require identification [20].

In recent years, computed tomography (CT) has been used to investigate human remains [21-28]. CT and three-dimensional reconstruction software with workstations are advantageous for cases where difficulties arise from maceration or if ethical concerns are raised for handling human remains (21, 25-28). Additionally, these tools are useful to retain accurate measurements and virtual data records. Morphometric analyses using CT images from living individuals with different clinical indications are very helpful for generating contemporary population-specific data [21-25, 28]. A study by Stull et al. [28] compared osteometric and virtual measurements of the same skeletal elements and confirmed that accurate measurements can be obtained from CT scan data. A number of sex estimation studies from the mandible [29], cranium [30], sternum [31], maxillar sinus [32], and femur [33] of the contemporary Turkish population have been published recently. There is one cadaveric study published on the tibia by Kirici and Ozan [ref] which is based on a very small sample (N=55) which makes the results questionable for forensic application.

Discriminant function analysis (DFA) is the most frequently used statistical method for classification by the researchers [34]. It has been used to produce population specific formulae for several different skeletal elements, including the tibia. Indeed, osteometric studies for sex estimation from the tibia have been conducted for several populations such as Northern Americans [11,12], medieval and modern Croatians [13,14] Portuguese [15], Southern Europeans [4], Czech [16] and Greek-Cypriots [17]. The high classification results (up to 95%) achieved in the abovementioned studies clearly makes the tibia a very successful sex indicator.

In the present study, we measured seven anthropometric parameters of the left tibia on virtual CT images. The first objective of this study was to provide population-specific sex estimation equations for the contemporary Turkish population based on CT images in a large enough sample to provide accurate and reliable estimations. A second objective was to test the sex estimation formulae on Southern Europeans by Kranioti and Apostol [4].

Material and methods

Sample description

The present study was conducted at the Tepecik Training and Research Hospital. All medical records and CT images of patients admitted to the different clinics of the hospital, from June 2014 and July 2016, were retrospectively evaluated. Cases that had fracture, surgery, congenital or an acquired anomaly in the tibia were excluded from the study (41 cases). The sample consists of 203 left tibia, 124 males and 79 females from Izmir, which is located in the South West of Turkey. Demographic information for the sample can be found in table 1. The study protocol was approved by the Tepecik hospital Ethics Board.

Data acquisition

All examinations were performed by a 64-slice CT scanner (Siemens Medical Solutions, Erlangen, Germany). A routine peripheral angiography multi-detector row computed tomography (MDCT) protocol was followed. The scanning parameters included 80 kV, 115 mAs, slice thickness 1mm and 512x512 matrix.

In preparation for the study readings, all multidetector CT angiography data were transf erred from the archive to a workstation (Aquarius Workstation; TeraRecon, San Mateo, CA) via internal network connections, providing 3D postprocessing options, multiplanar image reformatting (MPR), and maximum intensity projections.
CT scan data was used to create 3D reconstruction of the tibia and four measurements (ML, UB, LB and IntCondB) were taken on each bone (Fig 1 and 2). In addition, measurements related to the nutrient foramen (NFap, NFtrv and NFCirc) were taken on axial CT images (Fig 3 and 4). Each measurement was performed by researchers manually at the workstation. Description of each measurement can be found in table 2. Figures 1-4 illustrate the measurements.

Inter- and Intra-observer error was estimated in a sample of N=20 tibia using technical measurement error (TEM), relative TEM (rTEM) and coefficient of reliability (R) of the measurement. rTEM, which expresses the error as a percentage of TEM divided by the average value for each measurement, was also taken in order to scale the error. The coefficient R of the measurement is calculated as suggested by Ulijaszek and Kerr [35].

Validation of published formulae for Southern Europeans

Equations F1-F4 (Table 4) for Southern Europeans were tested using three measurements (ML, UB, LB) on this sample. Percentages of correct classification were calculated for males and females separately and for the pooled sample.

Data analysis

Variables were tested for normality and equal variances between the two groups (males and females) and parametric and non-parametric tests (e.g. ANOVA, Wilcoxon test) were used to explore if there are statistically significant differences between the sexes.

Univariate and multivariate discriminant function analysis was used to create population specific formulae for the Turkish population. Data analysis was done using SPSS 22.

Results

Inter- and Intra-observer error was estimated using technical measurement error (TEM), relative TEM (rTEM) and coefficient of reliability (R) of the measurement. The results are illustrated in table 3. Intra-observer error is low and inter-observer error is relatively higher. Interestingly the variable with the highest error in both cases is TUB with R=0.73 between two different observers.

Four equations based on all possible combinations of three variables of the tibia were published by Kranioti and Apostol [4] on a pooled Southern European sample consisting of populations from Spain, Italy, and Greece. Applying the formulae in our sample resulted in very high classification accuracy ranging from 81 to 88%. These results are only 0.1- 2% lower than the accuracy reported in the original study (see table 4). In addition, 36.5-47% of the total Turkish sample is correctly classified with over 95% posterior probability, which is actually higher than the one reported for the original sample (25-43%). Males gave higher accuracies for all formulae (50-60%) while female values were significantly lower with F3 giving the lowest accuracy (5.1%) with over 95% posterior probability of correct classification.

In addition to the validation of the formulae for Southern Europeans (SE) four more variables were tested for the modern Turkish population. Kolmogorov-Smirnof (K-S) and Shapiro Wilk (S-W) tests were used to test normal distribution of the data for each variable and for both groups. S-W test revealed 3 variables (LM, NFtrsv, NFcirc) that did not follow normal distribution in females. This however is not expected to cause significant problems in large samples (>40) [36]. To avoid any problems both ANOVA (with 1000 bootstraps) and Wilcoxon W (Monte-Carlo 2-tailed test based on 1000 subsamples) tests were used to explore differences between sexes. According to both tests mean differences for all variables were found to be statistically significant (P<0.001) between the sexes.

Univariate statistics

Univariate discriminant functions were created for all variables. The best single variable was found to be UB (86%) followed by LB (85%). NFcirc, ML and NFap gave accuracies slightly below 80% (Table 5). NFtrsv and IntCondB performed the worst with classification accuracies 74% and 66% respectively. The poor classification results for these variables deem them inappropriate for use as single sex indicators therefore they are omitted from table 5.

Multivariate statistics

Eight multivariate discriminant functions (TUR1-8) were created using different combinations of variables and considering fragmented models of the tibia (Table 6). Classification accuracy was calculated per group and in total for both original and cross-validated data. Classification accuracy for cross-validated data ranged from 79 to 86% and it was very close to the accuracy obtained for the original data in all cases. The best discriminant function (TUR1) used ML, UB and LB and resulted in 86.2% accuracy. Function F1 (Table 4) developed for the Southern European sample used the same variables and interestingly classified the Turkish sample with higher accuracy (87.7%). Function TUR3 which used ML and LB, classified 85% of the Turkish sample correctly while F3 for the Southern European sample (uses the same variables) correctly classified only 80% of the sample.

Discussion

Sexual dimorphism of the human skeleton is a powerful biological feature that can aid forensic investigations of unknown human remains to achieve positive identification. In the absence of a complete set of human remains forensic practitioners are tasked with estimating the biological profile of the individuals with single and often fragmented skeletal parts. Osteometric sex estimation methods are known to be population specific, thus in the past decades several osteometric studies produced standards for different bones and populations [9, 37-39]. Such studies are possible due to an increasing number of documented skeletal collections that are currently available around the world [37, 40,43]. This approach however is not feasible in several occasions, such as in Islamic countries where religion forbids the exhumation of human remains and the creation of modern skeletal collections. To overcome this problem medical imaging and sophisticated software for 3D modelling came into play in the past decade allowing for easy 3D object manipulation and quantification even of very small features, such as the space on the temporal bone that houses the labyrinth [44].

A large sample of CT scans of the left tibia (N=203) were used to acquire seven measurements from a population of South-West Turkey. As a first step the study sample was used to test 4 published formulae for a Southern European sample consisting of Italians, Spanish and Greeks [4]. Our sample comes from Izmir, on the Mediterranean part of Turkey and it actually applies to the broader context of “SE”/Mediterranean populations. These formulae use different combinations of 3 measurements (ML,UB,LB) and give classification accuracy 82.7-87.8%. The Turkish sample presents almost identical overall classification accuracy (80.8-87.2%) compared to the original study (Table 4). In addition, the Turkish sample is classified more successfully with over 95% posterior probability in all equations. For example, F1 classifies 47% of the Turkish with over 95% confidence compared to 43% that is noted in the original study (Table 4). These results create the impression that the SE formulae are sufficient for modern Turkish populations and they should be used unquestionably for forensic applications. Careful observation of the two subgroups however, reveals that males are more accurately classified compared to females. F3 and F4 correctly classify 93% and 91% of males compared to 60% and 76% of females respectively. Is this lack of balance important for the evaluation of the method?

A similar observation occurred in other studies, such as the one on metacarpals by Lazenby et al.[45] when he tested the Scheuer and Erlinghton [46] equations on a 19th Century Canadian sample or the validation study of Nathena et al. [47], when testing the formulae deriving from the Athens collection (mainland Greeks) on an islander sample from Crete. Disproportionately low classification in females means that upon application of the method in forensic settings true females have higher probability to be classified as males thus obscuring positive identification [47]. This indicates that balanced allocation accuracy for both sexes is more important than a higher overall sex allocation accuracy in forensic situations as noted by Khanpetch et al. [48]. Taking this under consideration we submitted the Turkish data to univariate and multivariate discriminant function analysis in order to obtain population specific standards. The best single variable was found to be UB (86%) followed by LB (85%). The best multivariate discriminant function (TUR1) used ML,UB and LB and resulted in 86.2% accuracy. This is actually slightly lower than F1 for the European sample that uses the same variables. Yet the classification accuracy is better balanced between the sexes in TUR1 and generally in all the multivariate formulae we created (see Table 6). In addition, TUR4 exhibits higher and more balanced classification accuracy then F4.

It is evident that the classification results for both the SE formulae are high and comparable with other studies [12,16]. F1 and F2 give relatively good balance between the sexes and a good proportion of the sample is correctly classified with over 95% confidence, thus it is safe to be used for identification of modern Turkish. F3 and F4 on the other hand, present disproportional accuracies that raise the danger of misclassification of females. All eight formulae for the Turkish sample present balanced accuracies between the sexes. In addition, TUR7 and TUR8 offer the opportunity to classify fragmented tibia, which are missing the upper or both epiphyses respectively.

The current study aspires to create population specific standards for sex estimation for the modern Turkish population in order to assist with positive identification of the numerous mass graves that are currently under investigation in the country. Yet, Turkey is a large country extending from the Mediterranean Sea deep into Asia with a rather complex social structure. History confirms that many different ethnic groups live in Turkey today including Turkish, Greeks and other Balkans populations. Socioeconomic and dietary differences between mainland, northern Turkey and the Mediterranean coast may naturally be reflected on biological differences and the expression of sexual dimorphism. The current study is a first effort to provide population specific standards, but one should consider that the origin of the study population is the Mediterranean coast of Izmir, and does not necessarily deem the results applicable to other regions of Turkey without further testing. This phenomenon however can explain the good performance of the SE formulae [4] for Turkish compared to the results reported by Kotěrová et al. [16] for Czechs.

Taking under consideration the principles of good practise in Forensic Anthropology it is recommended to use population specific formulae for sex estimation for any unidentified individuals suspected to be of Turkish ethnicity. Yet, since classification for F1 and F2 was higher than TUR1 and TUR2 it is very likely that not all morphological variation of the tibia is depicted by our sample, thus it would be preferable to use both methods and make a decision based on the posterior probability. F3 and F4 are not recommended to be used alone, unless posterior probability is strikingly high for any sex. When only fragments are available TUR7 and TUR8 should be used but in conjunction with any available other methods.

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Table 1. Summary table of the sample used in the study

Sex	Total	Mean age	SD	Minimum age	Maximum age
Male	124	59.81	12.20	19	82
Female	79	60.20	14.54	29	92

Table 2. Definitions of tibial measurements

Measurements	**Distance**
Maximum length (ML)	Distance from the superior articular surface of the lateral condyle to the tip of the medial malleolus.
Proximal Epiphyseal Breadth (UB)	Maximum distance between the two most laterally projecting points on the medial and lateral condyles of the proximal articular region (epiphysis).
Distal Epiphyseal Breadth (LB)	Maximum distance between the two most laterally projecting points on the medial malleolus and the lateral surface of the distal articular region (epiphysis).
Maximum Diameter at the Nutrient Foramen (NFap)	Distance between the anterior crest and the posterior surface at the level of the nutrient foramen
Medial-Lateral (Transverse) Diameter at the Nutrient Foramen (NFtrv)	Straight line distance of the medial margin from the interosseous crest at the level of the nutrient foramen
Circumference at the Nutrient Foramen (NFCirc)	Circumference measured at the level of the nutrient foramen.
Intercondylar breadth (IntCondB)	Distance between medial and lateral intercondylar eminence points

Table 3. Intra- and Inter-observer error is quantified by calculating TEM, rTEM and R for each variable.

	Intra-Observer Error (N=20)			Inter-Observer Error (N=20)		
	TEM	rTEM	R	TEM	rTEM	R
ML	1.49	0.42	1	2.41	0.69	0.99
UB	1.51	2.07	0.89	2.36	3.22	0.73
NFap	0.4	1.1	1	0.74	2.08	0.98
NFtrsv	0.14	0.56	1	0.4	1.62	0.97
NFCirc	1.57	1.62	0.97	3.4	3.52	0.88
LB	0.58	1.15	0.97	1.08	2.14	0.9
IntCondB	0.3	2.69	0.97	0.51	4.46	0.93

Table 4. Classification accuracy of the Turkish sample using the formulae F1-4 for a Southern European sample.

			Turkish						Southern Europeans-original sample			
			Males (N=124)		Females (N=79)		Total (N=203)		Males	Females	Total	
			>50% PP	>95% PP	>50% PP	>95% PI	>50 % PP	>95% PP	>50% PP	>50% PP	>50% PP	>95% PP
F1	0.0183*ML+0.169*TUB+0.0505*TLB-20.8371	N	114	74	64	21	178	95	181/208	200/226		
		%	91.2	59.7	81.0	26.6	87.7	46.8	87	88.5	87.8	43.3
F2	0.0196*ML+0.1890*TUB-20.458	N	111	62	66	25	177	87	183/209	198/231		
		%	89.5	50.0	83.5	31.6	87.2	42.9	87.6	85.7	86.6	39.3
F3	0.0372*ML+0.1213*TLB-18.3472	N	115	70	49	4	164	74	167/212	201/232		
		%	92.7	56.5	62	5.1	80.8	36.5	78.8	86.6	82.8	24.8
F4	0.2255*TUB+0.0543*TLB-18.7601	N	113	72	60	20	173	92	175/208	198/226		
		%	91.1	58.1	75.9	25.3	85.2	45.3	84.1	87.6	85.9	35

Table 5. Univariate statistics for tibial measurements, sectioning points and classification results.

		Original					Cross-validated				
	Demarking point	Males (N=124)		Females (N=79)		Total	Males (N=124)		Females (N=79)		Total
		N	%	N	%	%	N	%	N	%	%
ML	349.9	94	75.8	64	81	77.8	94	75.8	64	81	77.8
UB	73.2	107	86.3	69	87.3	86.7	107	86.3	69	87.3	86.7
NFap	30.4	96	77.4	62	78.5	77.8	96	77.4	62	78.5	77.8
NFCirc	94.4	100	80.6	61	77.2	79.3	100	80.6	61	77.2	79.3
LB	50.2	106	85.5	67	84.8	85.2	106	85.5	66	83.5	84.7

Table 6. Discriminant functions and classification accuracy for original and crossvalidated data.

									Original					Cross-validated				
									Male		Female		Total	Male		Female		Total
Functions	ML	UB	Nfap	NFtrsv	NFCirc	LB	IntCondB	Constant	N	%	N	%	%	N	%	N	%	%
TUR1	0.008	0.168				0.113		-20.703	107	86.3	69	87.3	86.7	107	86.3	68	86.1	86.2
TUR2	0.015					0.272		-18.966	105	84.7	67	84.8	84.7	105	84.7	67	84.8	84.7
TUR3	0.011	0.227						-20.576	106	85.5	67	84.8	85.2	106	85.5	67	84.8	85.2
TUR4		0.18				0.132		-19.845	106	85.5	69	87.3	86.2	105	84.7	69	84.7	85.7
TUR5	0.004	0.137	0.014	-0.078	0.03	0.13	0.083	-20.291	108	87.8	68	86.1	87.1	106	86.2	67	84.8	85.6
TUR6		0.213	-0.004	-0.039	0.038		0.083	-19.997	105	85.4	69	87.3	86.1	105	85.4	67	84.8	85.1
TUR7			0.084	-0.078	0.039	0.253		-17.44	107	87	67	84.8	86.1	104	84.6	65	82.3	83.7
TUR8			0.107	0.052	0.074			-12.03	101	82.1	62	78.5	99	80.5	80.7	61	77.1	79.2

Figure 1. Figure 1. Measurements of ML, UB and LB on 3D image

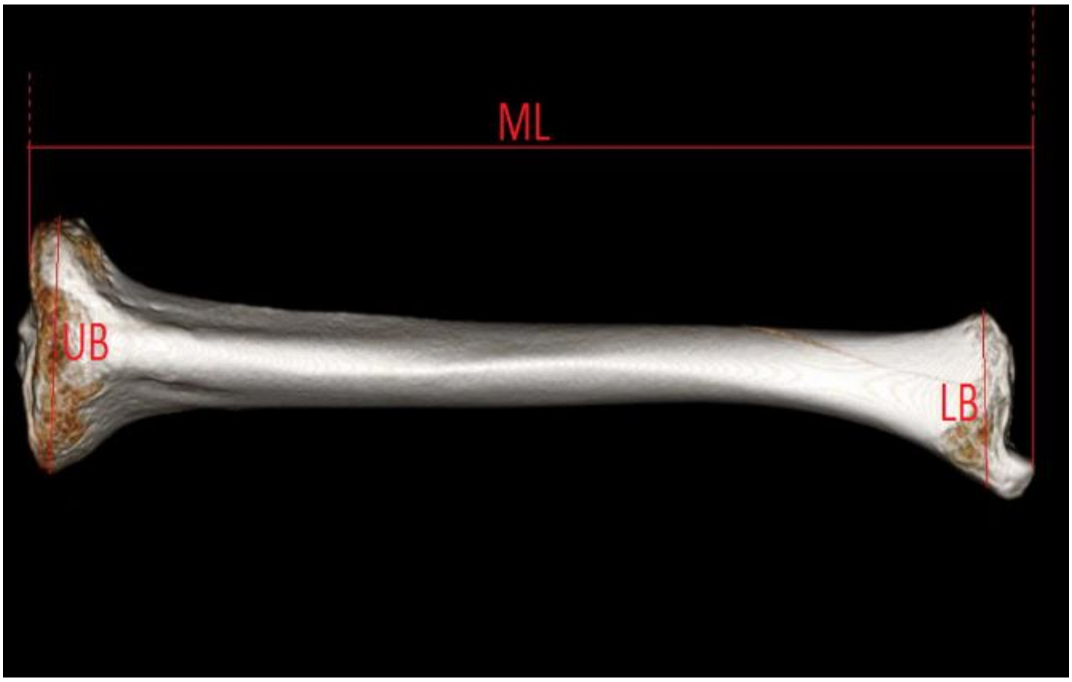

Figure 2. Measurements of IntCondB on 3D image

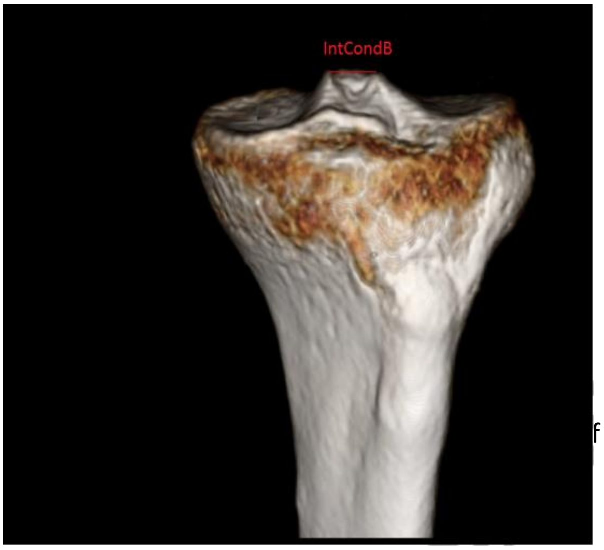

Figure 3. Measurements of NFap and NFtrv on axial CT image

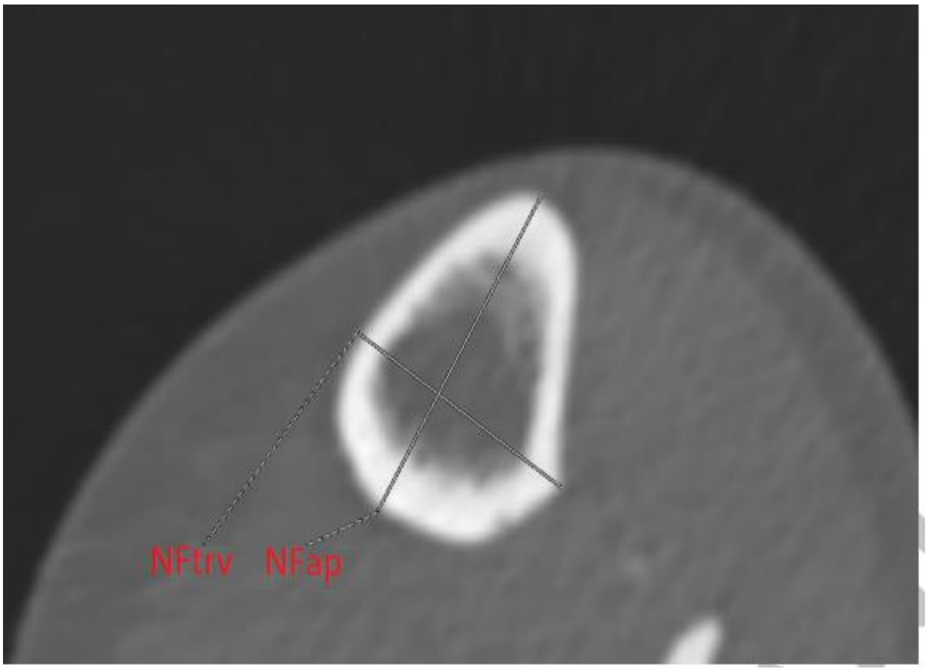

Figure 4. Measurements of NFCirc on axial CT image